

VERIFICATION OF TRANSLATION

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This on the 22<sup>nd</sup> day of June, 2010

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[Designation of Document] Request for Patent

[Reference Number] P36768

[Date of Submission] March 31, 2003

[Destination] Commissioner of the Patent Office

[International Patent Classification] C08F 12/08  
C08G 63/02  
C08G 63/06  
C08G 63/181  
C08G 64/04  
D04H 1/72  
D04H 3/00

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[Priority Claimed based on Prior Application]	
[Application Number]	Patent Application No. 2003-34779
[Application Date]	February 13, 2003
[Indication of Fee]	
[Deposit Account Number]	010250
[Amount of Payment]	¥21,000
[List of Submitted Article]	
[Name of Article]	Specification 1
[Name of Article]	Drawings 1
[Name of Article]	Abstract 1
[General Power of Attorney Number]	0203001
[Necessity of Proof]	Necessary

[Designation of Document] Specification

[Title of the Invention] POROUS FIBER, POROUS FIBER LAMINATE  
AND METHOD FOR PRODUCTION THEREOF

[Claims]

[Claim 1] A porous fiber comprising a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B), and having an average fiber diameter of 0.1-50  $\mu\text{m}$  and a void percentage of at least 5%.

[Claim 2] The porous fiber according to claim 1, wherein the hydrophobic solvent is a halogen element-containing hydrocarbon.

[Claim 3] The porous fiber according to claim 1 or 2, wherein the number-average molecular weight of the organic compound (B) is not less than 62 nor more than 300.

[Claim 4] The porous fiber according to any one of claims 1 to 3, wherein the hydrophobic solvent is at least one selected from the group consisting of methylene chloride, chloroform, dichloroethane, tetrachloroethane, trichloroethane, dibromomethane, and bromoform.

[Claim 5] The porous fiber according to any one of claims 1 to 4, wherein the hydrophobic solvent-soluble polymer (A) is at least one selected from the group consisting of polylactic acid, polycaprolactone, polycarbonates, polystyrene, and polyarylates.

[Claim 6] A fiber laminate comprising porous fibers

that comprise a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B), and have an average fiber diameter of 0.1-50  $\mu\text{m}$  and a void percentage of at least 5%.

[Claim 7] The fiber laminate according to claim 6, wherein the hydrophobic solvent is a halogen element-containing hydrocarbon.

[Claim 8] The fiber laminate according to claim 6 or 7, wherein the number-average molecular weight of the organic compound (B) is not less than 62 nor more than 300.

[Claim 9] The fiber laminate according to any one of claims 6 to 8, wherein the hydrophobic solvent is at least one selected from the group consisting of methylene chloride, chloroform, dichloroethane, tetrachloroethane, trichloroethane, dibromomethane, and bromoform.

[Claim 10] The fiber laminate according to any one of claims 6 to 9, wherein the hydrophobic solvent-soluble polymer (A) is at least one selected from the group consisting of polylactic acid, polycaprolactone, polycarbonates, polystyrene, and polyarylates.

[Claim 11] A method for producing the fiber laminate according to any one of claims 6 to 10, including a step in which a solution is produced by dissolving a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B) in a hydrophobic solvent, a

step in which the solution is spun by electrospinning, and a step in which a fiber laminate accumulated on a collector is obtained.

[Claim 12] The method for producing the fiber laminate according to claim 11, wherein the hydrophobic solvent is a halogen element-containing hydrocarbon.

[Claim 13] The method for producing the fiber laminate according to claim 11 or 12, wherein the hydrophobic solvent is at least one selected from the group consisting of methylene chloride, chloroform, dichloroethane, tetrachloroethane, trichloroethane, dibromomethane, and bromoform.

[Detailed Description of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to a porous fiber mainly comprising a hydrophobic solvent-soluble polymer and an organic compound containing a plurality of hydroxyl groups, a fiber laminate, and a method for producing the same. More specifically, the present invention relates to a porous fiber formed without needing a coagulating solution, a fiber and a fiber laminate made of the same, and a method for producing the same.

[0002]

[Prior Art]

In tissue engineering fields, a porous structure is

sometimes used as a matrix when cells are cultured. As the porous structure, a freeze dried foamed body and a fiber structure are known (see, for example, non-patent literature 1). These porous structures must have affinity to cells, biodegradability, safety, and so on.

[0003]

Polyglycolic acid, which is used for a surgical suture etc., has excellent biocompatibility, biodegradability, and safety, and a fiber structure made of polyglycolic acid is being studied for using as the matrix (see, for example, non-patent literature 1). However, fiber diameters of the fiber structures obtained by using conventional methods are too large, and areas on which cells can be adhered are not sufficient. In order to increase the surface areas, fiber structures having finer fiber diameters are desired.

[0004]

On the other hand, as a manufacturing process for a fiber structure having finer fiber diameters, electrospinning is known (see, for example, patent literature 1 and 2). The process for electrospinning comprises steps introducing a liquid, for example, a solution containing a fiber-forming material, into an electric field to draw the liquid toward an electrode, and forming a fibrous material. Usually, the fiber-forming material is hardened during the solution is drawn. The hardening is performed, for example, by means of

cooling (e.g., in cases where the solution to be spun is solid at room temperature), chemical hardening (e.g., a treatment with a hardening vapor), solvent vaporization, and so on. Further, the obtained fibrous material is collected on an adequately placed receptor from which it can be removed if necessary. Furthermore, electrospinning can obtain a non-woven fibrous material directly, and thereby the operation of the process is simple and easy, that is, there is no need to form fibers once and then to form a fiber structure further.

[0005]

It is known to use a fiber structure obtained with electrospinning as a matrix for culturing cells. For example, it is currently under investigation to form a fiber structure from polylactic acid by using electrospinning and to regenerate blood vessels by culturing smooth muscle cells on the fiber structure (see, for example, non-patent literature 2). However, the fiber structures obtained by using electrospinning are apt to take a dense structure having short distances between fibers since the fibers have fine diameters. When the fiber structure is used as a cell culture matrix (scaffold), proliferated cells accumulate on the surfaces of each fibers that form the fiber structure, the surfaces of the fiber are covered thickly with progressing culture. Resultingly, it becomes difficult for a solution containing nutrients etc. to move sufficiently into inner parts of the



fiber structure, and this causes a state in which only sections near the surface of the fiber structure can afford to carry out cell culture.

[0006]

As a means enabling a solution containing nutrients etc. to move easily into the outermost surface of fibers which form the fiber structure during cell culture, it is proposed a means to make the fibers themselves, which form the fiber structure, porous fibers. For example, there was a report of electrospinning a fiber structure having regular pores on fiber surfaces from a volatile solvent-containing solution (see non-patent literature 3 and patent literature 3). However, by using this method, a fiber structure formed has pores only on fiber surfaces, and it is difficult to make porous so far as the inner fibers.

[0007]

Further, a method that forms porous fibers by electrospinning a fiber structure from a solution that contains a hydrophilic polymer and a hydrophobic polymer, and extracts the hydrophilic polymer by immersing the obtained fiber structure into water also has been reported (see non-patent literature 4 and patent literature 3). However, the operation of this method is complicated since a water-dipping treatment is needed. Further, the finally obtained porous fibers are substantially composed only of the

hydrophobic polymer, and the hydrophilicity of the fiber structure can not be controlled.

[0008]

[Patent literature 1]

JP-A 63-145465 (JP-A means Japanese unexamined patent publication).

[0009]

[Patent literature 2]

JP-A 2002-249966.

[0010]

[Patent literature 3]

The pamphlet of International Publication No. 02/16680,

[0011]

[Non-patent literature 1]

Tissue Engineering, page 258, the translation was supervised by the team represented by Noriya Oono and Masuo Aizawa, NTS INC., Jan. 31, 2002.

[0012]

[Non-patent literature 2]

Joel D. Stitzel, Kristin J. Pawlowski, Gary E. Wnek, David G. Simpson, and Gary L. Bowlin, Journal of Biomaterials Applications 2001, Vol. 16, USA, page 22-33.

[0013]

[Non-patent literature 3]

Michael Bognitzki, Wolfgang Czado, Thomas Frese, Andreas Schaper, Michael Hellwig, Martin Steinhart, Andreas Greiner, and Joachim H. Wendroff, Advanced Materials 2001, Vol. 13, USA, page 70-72.

[0014]

[Non-patent literature 4]

Michael Bognitzki, Thomas Frese, Martin Steinhart, Andreas Greiner, and Joachim H. Wendroff, Polymer Engineering and Science 2001, Vol. 41, USA, page 982-989.

[0015]

[Problems that the Invention is to Solve]

It is an object of the present invention to provide a material suitable for a matrix of cell culture in the field of tissue engineering and specifically to provide fibers and a fiber laminate in which a solution containing nutrients etc. needed for the cell culture can move easily into the whole portion of cells. Concretely, it is considered that the cell culture can be continued for a long period of time thereby. In order to achieve the object, it is considered that the fibers and fiber laminate are required to have large surface areas and large voids. Preferably, hydrophilicity is imparted, if necessary. At the same time, it is another object of the present invention to provide a production method of a hydrophilicity-imparted porous fiber laminate without needing complicated processes such as extraction and the like.

[0016]

[Means for Solving the Problems]

The present invention is as the following.

1. A porous fiber comprising a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B), and having an average fiber diameter of 0.1-50  $\mu\text{m}$  and a void percentage of at least 5%.
2. The porous fiber according to item 1., wherein the hydrophobic solvent is a halogen element-containing hydrocarbon.
3. The porous fiber according to item 1 or 2, wherein the number-average molecular weight of the organic compound (B) is not less than 62 nor more than 300.
4. The porous fiber according to any one of items 1 to 3, wherein the hydrophobic solvent is at least one selected from the group consisting of methylene chloride, chloroform, dichloroethane, tetrachloroethane, trichloroethane, dibromomethane, and bromoform.
5. The porous fiber according to any one of items 1 to 4, wherein the hydrophobic solvent-soluble polymer (A) is at least one selected from the group consisting of polylactic acid, polycaprolactone, polycarbonates, polystyrene, and polyarylates.
6. A fiber laminate comprising porous fibers that comprise a hydrophobic solvent-soluble polymer (A) and an organic

compound having a plurality of hydroxyl groups (B), and have an average fiber diameter of 0.1-50  $\mu\text{m}$  and a void percentage of at least 5%.

7. The fiber laminate according to item 6, wherein the hydrophobic solvent is a halogen element-containing hydrocarbon.

8. The fiber laminate according to item 6 or 7, wherein the number-average molecular weight of the organic compound (B) is not less than 62 nor more than 300.

9. The fiber laminate according to any one of items 6 to 8, wherein the hydrophobic solvent is at least one selected from the group consisting of methylene chloride, chloroform, dichloroethane, tetrachloroethane, trichloroethane, dibromomethane, and bromoform.

10. The fiber laminate according to any one of items 6 to 9, wherein the hydrophobic solvent-soluble polymer (A) is at least one selected from the group consisting of polylactic acid, polycaprolactone, polycarbonates, polystyrene, and polyarylates.

11. A method for producing the fiber laminate according to any one of items 6 to 10, including a step in which a solution is produced by dissolving a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B) in a hydrophobic solvent, a step in which the solution is spun by electrospinning, and a step in which a fiber

laminate accumulated on a collector is obtained.

12. The method for producing the fiber laminate according to item 11, wherein the hydrophobic solvent is a halogen element-containing hydrocarbon.

13. The method for producing the fiber laminate according to claim 11 or 12, wherein the hydrophobic solvent is at least one selected from the group consisting of methylene chloride, chloroform, dichloroethane, tetrachloroethane, trichloroethane, dibromomethane, and bromoform.

[0017]

[Mode for Carrying out the Invention]

Hereafter, the present invention will be explained in detail. In the present invention, the fiber laminate expresses a three-dimensional structure that are formed with heaping, weaving, knitting, or another method from single or a plurality of obtained fibers. Concrete forms of the fiber laminate include, for example, nonwoven fabrics. Further, tubes, meshes, and the like that are obtained by processing the above mentioned fiber laminate can be preferably used in the field of tissue engineering.

[0018]

Porous fibers and fiber laminates of the present invention contain a hydrophobic solvent-soluble polymer (A).

[0019]

The hydrophobic solvent of the present invention

expresses an organic substance that can not dissolve water of 5% or more in concentration at normal temperature (for example, 27°C) and is liquid at the temperature. As the hydrophobic solvent of the present invention, halogen element-containing hydrocarbons are preferable because they can dissolve polymers well. Examples of more preferable hydrophobic solvents are methylene chloride, chloroform, dichloroethane, tetrachloroethane, trichloroethane, dibromomethane, bromoform, and the like, and methylene chloride is especially preferable as the hydrophobic solvent. Among these solvents, volatile solvents are also preferably used. The volatile solvent expresses an organic substance that has a boiling point not higher than 200°C at atmospheric pressure and is liquid at normal temperature (for example, 27°C).

[0020]

"Soluble" in the present invention means that a solution containing a polymer of 1 wt% exists stably at normal temperature (for example, at 27°C) without forming precipitates. Examples of the hydrophobic solvent-soluble polymer (A) are polylactic acid, polylactic acid-polyglycolic acid copolymers, aliphatic polyesters such as polycaprolactone, polycarbonates, polystyrene, polyarylates, polymethylmethacrylate, polyethylmethacrylate, cellulose diacetate, cellulose triacetate, polyvinyl acetate, polyvinyl methyl ether, poly(N-vinylpyrrolidone), polybutylene

succinate, and polyethylene succinate, and copolymers of these polymers and the like. Among these polymers, as the hydrophobic solvent-soluble polymer (A), polylactic acid, polycaprolactone, polycarbonates, polystyrene, and polyarylates are preferable.

[0021]

Porous fibers and fiber laminates of the present invention may contain only one kind, or two or more kinds of the hydrophobic solvent-soluble polymer (A).

[0022]

Porous fibers and fiber laminates of the present invention contain an organic compound having a plurality of hydroxyl groups (B). When an organic compound that does not have a plurality of hydroxyl groups is used, the objective porous fibers can not be obtained, further a fiber laminate comprising the porous fibers can not be obtained stably, and furthermore, the cell culture becomes difficult in some cases, which is not preferable.

[0023]

It is preferable that the organic compound (B) has a number-average molecular weight not less than 62 nor more than 300. It is not preferable that the number-average molecular weight is more than 300 because the forming of porous fibers is difficult. Further, an example of the organic compound having a molecular weight of 62 and a plurality of hydroxyl



groups is ethylene glycol, and an organic compound having a molecular weight of less than 62 and a plurality of hydroxyl groups does not exist actually. More preferable number-average molecular weights of the organic compound (B) are not less than 62 nor more than 250.

[0024]

Examples of the organic compounds (B) include ethylene glycol, propylene glycol (1,2-propanediol), 1,3-propanediol, diethylene glycol, triethylene glycol, glycerin, pentaerythritol, polyethylene glycol, polypropylene glycol, polyethylene glycol-polypropylene glycol block polymers, and the like.

[0025]

In the present invention, polymers or compounds other than the hydrophobic solvent-soluble polymers (A) and the organic compounds having a plurality of hydroxyl groups (B) can be combinedly used (for example, copolymers of polymers, polymer blends, or compound mixtures) in a range where the purposes are not impaired.

[0026]

Porous fibers and fiber laminates of the present invention are formed of porous fibers having an average fiber diameter of 0.1-50  $\mu\text{m}$ . The average fiber diameter of less than 0.1  $\mu\text{m}$  is not preferable because such porous fibers and fiber laminates decompose too quickly in vivo when they are used as

cell culture matrices for tissue engineering. Further, the average fiber diameter of larger than 50  $\mu\text{m}$  is not preferable because the area on which cells can adhere becomes small. A more preferable average fiber diameter is 0.2-25  $\mu\text{m}$ , and an especially preferable average fiber diameter is 0.2-20  $\mu\text{m}$ . The fiber diameter expresses the diameter of a fiber cross-section when the cross-section is circular. However, sometimes the shape of a fiber cross-section is oval. The fiber diameter of this case expresses the value calculated by averaging the lengths of the major axis and the minor axis of the oval. Further, when the cross-section of the fiber is not circular nor oval, it is approximated to a circle or an oval, and the fiber diameter is calculated.

[0027]

Further, porous fibers of the present invention express fibers having isolated holes and/or continuous holes on surfaces and in inner parts of fibers. The isolated holes and/or the continuous holes of the inner parts of fibers may form empty bodies so that the fibers may be hollow fibers as a whole.

Fiber laminates of the present invention comprise porous fibers having a void percentage of 5% at least. The void percentage means that, on a fiber cross-section formed by cutting at an arbitrary position, the sum of the areas of the

isolated holes and the continuous holes that reach to the fiber surface, and the areas of the isolated holes and the continuous holes existing in the inner parts of the fiber, that is, the sum of the space areas where fiber forming substances (hydrophobic solvent-soluble polymers (A), organic compounds (B), and other necessary polymers and compounds) are absent, occupies at least 5% of the whole area of the fiber cross-section including the space areas. A void percentage of less than 5% is not preferable since a solution that contains nutrients and the like does not penetrate sufficiently into the inner parts of the matrix during cell culture. The void percentage is preferably 10% or more.

[0028]

That is, a preferable embodiment of the present invention is porous fibers comprising an aliphatic polyester (A) and an organic compound having a plurality of hydroxyl groups, and having an average fiber diameter of 0.1-50  $\mu\text{m}$  and a void percentage of at least 5%, and fiber laminates made therefrom. It is also a preferable embodiment of the present invention to use, in stead of the aliphatic polyester, a polycarbonate, polystyrene, or a polyarylate.

[0029]

There is no particular limitation of a method for producing fiber laminates of the present invention as long as it can produce fibers and the like having the above-mentioned

fiber diameters, but electrospinning is preferable. Hereafter, the fabrication method using electrospinning will be explained in detail.

[0030]

In electrospinning used in the present invention, a solution prepared by dissolving a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B) in a hydrophobic solvent is discharged into an electrostatic field generated between electrodes, the solution is drawn toward an electrode, and the formed fibrous material is accumulated on a collector, and thereby a fiber laminate can be obtained. That is, when the fibrous material is accumulated, porous fibers of the present invention are already formed. The fibrous material expresses not only a material having a state of porous fibers and fiber laminates in which the solvent of the solution is already evaporated, but also a material having a state in which the solvent of the solution is contained.

[0031]

At first, an apparatus to be used in electrospinning will be explained. The electrode to be used in the present invention may be arbitrarily selected from metals, inorganic materials, and organic materials as long as it exhibits conductivity. Further, it may be a body formed by placing a thin film of a metal, an inorganic substance, or an organic

substance that shows electroconductivity on an insulator. The electrostatic field of the present invention is formed between a pair of electrodes or among a plurality of electrodes, and a high voltage can be applied on any electrode. This includes the case where three electrodes in total consisting of two electrodes of different high voltages (for example, 15 kV and 10 kV) and an electrode connected to a ground are used, and the case where more than three electrodes are used is also included.

[0032]

Next, the fabrication method of the present invention using electrospinning will be explained in detail. In the first step, a solution is produced by dissolving a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B) in a volatile solvent. In the solution of the fabrication method of the present invention, the concentration of the hydrophobic solvent-soluble polymer (A) is preferably 1-30 wt%. The concentration of the hydrophobic solvent-soluble polymer (A) of less than 1 wt% is not preferable because the formation of fiber laminates becomes difficult due to extremely low concentration. Further, the concentration of more than 30 wt% is not preferable because fiber diameters of the obtained fiber laminate become large. The concentration of the hydrophobic solvent-soluble polymer (A) is more preferably 2-20 wt%.

[0033]

It is preferable that the concentration of the organic compound (B) in the solution of the present invention is 2-50 wt%. It is not preferable that the concentration of the organic compound (B) is less than 2 wt% since the total areas of the recessed parts and the void parts on fiber cross-sections become small. Further, the concentration of more than 50 wt% is not preferable since the formation of fiber laminates becomes difficult. The concentration of the organic compound (B) is more preferably 4-30 wt%.

[0034]

When the boiling point of the organic compound (B) used in the present invention is low, a part of the compound sometimes evaporates together with a solvent during spinning by means of electrospinning. In the present invention, it is preferable that at least 1 wt% or more of the organic compound (B) supplied remains in the product. The content is more preferably 5-60 wt%, and further preferably, 10-60 wt%.

[0035]

In the fabrication method of fiber laminates using electrospinning in the present invention, the hydrophobic solvent can be used alone, or a plurality of the volatile solvents can be used in combination. Further, in the present invention, the hydrophobic solvent can be used in combination with another kind of solvent in a range where the present object

is not missed. The concrete examples of the hydrophobic solvent are already shown above.

[0036]

Next, the step in which the above mentioned solution is spun by electrospinning will be explained. For discharging the solution into an electrostatic field, an arbitrary method can be used. For example, one example will be explained by using FIG. 1, hereinafter. The solution 2 is supplied to the nozzle, the solution is placed at a proper position in the electrostatic field, the solution is drawn from the nozzle by means of the electrostatic field, and thereby fibers are formed from the solution. For this purpose, an appropriate apparatus can be used. For example, an appropriate means, e.g., an injection needle-shaped solution ejection nozzle 1 on which voltage is applied by using a high voltage generator 6 is placed at the tip of a cylindrical solution-holding tank 3 of a syringe, and the solution is guided to the tip. The tip of the ejection nozzle 1 is placed at an appropriate distance from a grounded fibrous material-collection electrode 5, and when the solution 2 comes out from the tip of the ejection nozzle 1, a fibrous material is formed between the tip and the fibrous material-collection electrode 5.

[0037]

Further, fine drops of the solution can be introduced into the electrostatic field with a method known by those in

the art, and one example of the method will be explained by using FIG. 2, hereinafter. The only requirement in this example is that a droplet is held in the electrostatic field apart from the fibrous material-collection electrode 5 at a distance in which fibers can be formed. For example, an electrode 4 which directly opposes the fibrous material-collection electrode may be directly inserted into the solution 2 in a solution-holding tank 3 having a nozzle 1.

[0038]

When the solution is fed into the electrostatic field from nozzle, a plurality of nozzles can be used so that the production rate of the fibrous material increases. The distance between the electrodes depends on an electrostatic charge level, the size of a nozzle, the flow amount and the concentration of the solution to be spun, and the like, but the distance of 5-20 cm was appropriate with approximately 10 kV. Further, an impressed electrostatic voltage is generally 3-100 kV, preferably 5-50 kV, and more preferably 5-30 kV. The desired voltage can be generated by using an arbitrary appropriate method.

[0039]

The above-mentioned explanation concerns the case where an electrode concurrently works also as a collector; but a collector may be provided aside from the electrode by placing



a material that can serve as a collector between electrodes so that a fiber laminate is collected on it. In this case, for example, a belt-shaped material is placed between electrodes, and by using the material as the collector, continuous production becomes possible.

[0040]

Finally, a step in which the fiber laminate accumulated on a collector is obtained will be explained. In the present invention, while the solution is drawn toward the collector, the solvent evaporates according to conditions, and a fibrous material is formed. The solvent evaporates completely before the fibrous material is collected on the collector at ordinary room temperature; however, if the evaporation of the solvent is not sufficient, the solution may be drawn under reduced pressure. At the latest, when the fibrous material is collected on the collector, porous fibers of the present invention are formed. Further, the temperature for drawing the solution depends on the evaporation behavior of the solvent and the viscosity of the solution to be spun; however, it is usually 0-50°C. The porous fibers are further accumulated over themselves on the collector, and thereby a fiber laminate of the present invention is produced.

[0041]

That is, a preferable embodiment of fabrication methods of the present invention is a fabrication method which includes

a step in which a solution is produced by dissolving an aliphatic polyester (A) and an organic compound having a plurality of hydroxyl groups (B) in a volatile solvent, a step in which the solution is spun by using electrospinning, and a step in which a fiber laminate accumulated on a collector is obtained; and the fiber laminate comprising porous fibers having an average fiber diameter of 0.1-50  $\mu\text{m}$  and a void percentage of at least 5% is obtained. It is also a preferable embodiment of the present invention to use, in stead of the aliphatic polyester, a polycarbonate, polystyrene, or a polyarylate, and the above-mentioned hydrophobic solvent may be used in stead of the volatile solvent.

[0042]

A fiber laminate obtained in the present invention can be used alone; however, it may be used in combination with another member with due regard to handleability and other requirements. For example, a nonwoven fabric, a woven fabric, a film, or the like that can serve as a supporting material is used as the collector, and a fiber laminate is formed on it; and thereby a member in which a supporting material and the fiber laminate are combined can be prepared.

[0043]

Uses of fiber laminates obtained in the present invention are not limited to cell culture matrices for tissue engineering, but they can be used as various applications such as all sorts

of filters, catalyst supporting materials, or the like in which special features of the present invention, that is, dent parts and voids, are used practically.

[0044]

[Advantage of the Invention]

The present invention is to provide a fiber laminate comprising porous fibers having small fiber diameters, and having dent parts and voids on the surface of fibers and in the inner cross-section thereof, and a production method thereof. Further, the fiber laminate obtained by the present invention has a very large surface area and a large number of voids, so that when the fiber laminate is used as a matrix of cell culture, cells easily grow. Furthermore, when an aliphatic polyester, which is a typical example of polymers having degradability and absorbability in vivo, is particularly used as a polymer to form the fiber laminate of the present invention, the fiber laminate exhibits very favorable characteristics such that it has an excellent degradability and absorbability in vivo, and when used as a matrix of cell culture for tissue engineering application, the matrix as such can be embedded in vivo.

[0045]

Further, the fiber laminate obtained by the present invention has a very large surface area and a large number of voids, so that when used as all sorts of filters, catalyst

supporting materials, the fiber laminate exhibits very favorable characteristics such as the remarkably enhanced efficiency thereof.

[0046]

[Examples]

The present invention will be explained hereafter with examples, while the present invention is not restricted by the examples. Further, evaluations in each of the following examples and comparative examples were carried out according to the following methods.

[0047]

[Void Percentage]

Scanning electron micrograph image depicting fiber cross-sections of obtained porous fibers or fiber laminates (at a magnification of  $\times 10,000$ ) were photographed. The whole part of the fiber cross-section was cut out from the photograph paper of the cross-section photograph image, and its weight was measured. Subsequently, the void parts of the fiber were cut out from the photograph paper, and their weights were measured. The void percentage of a fiber was calculated from these weights. This process was repeated for  $n=5$ , and the average value was calculated.

[0048]

[Average Fiber Diameter]

Scanning electron micrograph image depicting surfaces

of obtained porous fibers or fiber laminates (at a magnification of  $\times 2,000$ ) were photographed, and fiber diameters were measured from the photograph image for  $n=20$ , thereby calculating the average value.

[0049]

[Method for Determining Organic Compound (B)]

$^1\text{H}$ -NMR spectrum of an obtained fiber laminate was taken with EX-270 manufactured by JEOL Ltd. at  $20^\circ\text{C}$  using deuteriochloroform ( $\text{CDCl}_3$ ) as a solvent. The molar ratio in the fiber laminate was determined from the integration ratio of protons which were derived from the chemical structure of the polymer used and that of the organic compound (B) used, and the content (wt%) of the organic compound was calculated based on the molar ratio.

[0050]

[Example 1]

One pt. wt. of polylactic acid (trade mark "Lacty 9031", manufactured by Shimazu Seisakusyo), 1 pt. wt. of ethylene glycol (special grade chemical, manufactured by Wako Pure Chemical Industries, Ltd.), and 8 pts. wt. of methylene chloride (special grade chemical, manufactured by Wako Pure Chemical Industries, Ltd.) were mixed at room temperature ( $25^\circ\text{C}$ ) to prepare a slightly milky solution. The solution was discharged over 5 minutes toward a fibrous material-collection electrode 5 by using an apparatus shown in FIG. 2. The inside

diameter of the ejection nozzle 1 was 0.8 mm, the voltage was 12 kV, and the distance from the ejection nozzle 1 to the fibrous material-collection electrode 5 was 10 cm. The obtained fiber laminate was measured by using a scanning electron microscope (S-2400, manufactured by Hitachi). The average fiber diameter was 3  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 40%, and the ethylene glycol content in the fiber laminate was 18.0 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 3 and FIG. 4.

[0051]

[Example 2]

Except that 1 pt. wt. of diethylene glycol (special grade chemical, manufactured by Wako Pure Chemical Industries, Ltd.) was used in stead of the ethylene glycol, the same operations as Example 1 were carried out. The average fiber diameter was 4  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 15%, and the diethylene glycol content in the fiber laminate was 47.9 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 5 and FIG. 6.

[0052]

[Example 3]

Except that 1 pt. wt. of triethylene glycol (first class chemical, manufactured by Wako Pure Chemical Industries, Ltd.) was used in stead of the ethylene glycol, the same operations as Example 1 were carried out. The average fiber diameter was 3  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 15%, and the triethylene glycol content in the fiber laminate was 46.2 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 7 and FIG. 8.

[0053]

[Example 4]

Except that 1 pt. wt. of polyethylene glycol (average molecular weight of 200, first class chemical, manufactured by Wako Pure Chemical Industries, Ltd.) was used in stead of the ethylene glycol, the same operations as Example 1 were carried out. The average fiber diameter was 2  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 15%, and the polyethylene glycol content in the fiber laminate was 50.0 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 9 and FIG. 10.

[0054]

[Example 5]

Except that 1 pt. wt. of propylene glycol (1,2-propanediol) (special grade chemical, manufactured by Wako Pure Chemical Industries, Ltd.) was used in stead of the ethylene glycol, the same operations as Example 1 were carried out. The average fiber diameter was 4  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 15%, and the 1,2-propanediol content in the fiber laminate was 15.3 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 11 and FIG. 12.

[0055]

[Example 6]

Except that 1 pt. wt. of polycaprolactone (average molecular weight of approximately 70,000-100,000, manufactured by Wako Pure Chemical Industries, Ltd.) was used in stead of the polylactic acid, the same operations as Example 1 were carried out. The average fiber diameter was 4  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 15%, and the ethylene glycol content in the fiber laminate was 16.7 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 13 and FIG. 14.

[0056]

[Example 7]

Except that 1 pt. wt. of polycarbonate (trade mark of



"Panlite L1250", manufactured by Teijin Chemicals Ltd.) was used in stead of the polylactic acid, the same operations as Example 1 were carried out. The average fiber diameter was 3  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 35%, and the ethylene glycol content in the fiber laminate was 12.3 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 15 and FIG. 16.

[0057]

[Example 8]

Except that 1 pt. wt. of polystyrene (average molecular weight of 250,000, manufactured by Kanto Chemicals Ltd.) was used in stead of the polylactic acid, the same operations as Example 1 were carried out. The average fiber diameter was 6  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 35%, and the ethylene glycol content in the fiber laminate was 11.2 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 17 and FIG. 18.

[0058]

[Example 9]

Except that 1 pt. wt. of polyarylate (trade mark of "U-polymer U-100", manufactured by Yunitika) was used in stead

of the polylactic acid, the same operations as Example 1 were carried out. The average fiber diameter was 3  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. The void percentage was approximately 35%, and the ethylene glycol content in the fiber laminate was 12.5 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 19 and FIG. 20.

[0059]

[Comparative Example 1]

Except that 9 pt. wt. of methylene chloride was used in stead of the ethylene glycol, the same operations as Example 1 were carried out. The average fiber diameter was 2  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. Dent parts and void parts were not observed in fiber cross-sections, and hence the void percentage was 0%. The content of the organic compound (B) in the fiber laminate was 0 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 21 and FIG. 22.

[0060]

[Comparative Example 2]

Except that 1 pt. wt. of polyethylene glycol (average molecular weight of 400, first class chemical, manufactured by Wako Pure Chemical Industries, Ltd.) was used in stead of the ethylene glycol, the same operations as Example 1 were

carried out. The average fiber diameter was 3  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. Dent parts and void parts were not observed in fiber cross-sections, and hence the void percentage was 0%. The content of polyethylene glycol in the fiber laminate was 50.0 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 23 and FIG. 24.

[0061]

[Comparative Example 3]

Except that 1 pt. wt. of polyethylene glycol (average molecular weight of 600, first class chemical, manufactured by Wako Pure Chemical Industries, Ltd.) was used in stead of the ethylene glycol, the same operations as Example 1 were carried out. The average fiber diameter was 3  $\mu\text{m}$ , and fibers having a fiber diameter of 20  $\mu\text{m}$  or more were not detected. Dent parts and void parts were not observed in fiber cross-sections, and hence the void percentage was 0%. The content of polyethylene glycol in the fiber laminate was 50.0 wt%. Scanning electron micrographs of a surface and a fiber cross-section of the fiber laminate are shown in FIG. 25 and FIG. 26.

[Brief Description of the Drawings]

[FIG. 1]

FIG. 1 is one example of the apparatus to be used in

electrospinning, in which the solution to be spun is discharged into an electrostatic field, in the production method of the present invention.

[FIG. 2]

FIG. 2 is one example of the apparatus to be used in electrospinning, in which fine drops of the solution to be spun is introduced into an electrostatic field, in the production method of the present invention.

[FIG. 3]

FIG. 3 is the surface of the fiber laminate obtained in Example 1 (at a magnification of  $\times 2,000$ ).

[FIG. 4]

FIG. 4 is the cross-section of the fiber obtained in Example 1 (at a magnification of  $\times 10,000$ ).

[FIG. 5]

FIG. 5 is the surface of the fiber laminate obtained in Example 2 (at a magnification of  $\times 2,000$ ).

[FIG. 6]

FIG. 6 is the cross-section of the fiber obtained in Example 2 (at a magnification of  $\times 10,000$ ).

[FIG. 7]

FIG. 7 is the surface of the fiber laminate obtained in Example 3 (at a magnification of  $\times 2,000$ ).

[FIG. 8]

FIG. 8 is the cross-section of the fiber obtained in

Example 3 (at a magnification of  $\times 10,000$ ).

[FIG. 9]

FIG. 9 is the surface of the fiber laminate obtained in Example 4 (at a magnification of  $\times 2,000$ ).

[FIG. 10]

FIG. 10 is the cross-section of the fiber obtained in Example 4 (at a magnification of  $\times 10,000$ ).

[FIG. 11]

FIG. 11 is the surface of the fiber laminate obtained in Example 5 (at a magnification of  $\times 2,000$ ).

[FIG. 12]

FIG. 12 is the cross-section of the fiber obtained in Example 5 (at a magnification of  $\times 10,000$ ).

[FIG. 13]

FIG. 13 is the surface of the fiber laminate obtained in Example 6 (at a magnification of  $\times 2,000$ ).

[FIG. 14]

FIG. 14 is the cross-section of the fiber obtained in Example 6 (at a magnification of  $\times 10,000$ ).

[FIG. 15]

FIG. 15 is the surface of the fiber laminate obtained in Example 7 (at a magnification of  $\times 2,000$ ).

[FIG. 16]

FIG. 16 is the cross-section of the fiber obtained in Example 7 (at a magnification of  $\times 10,000$ ).

[FIG. 17]

FIG. 17 is the surface of the fiber laminate obtained in Example 8 (at a magnification of  $\times 2,000$ ).

[FIG. 18]

FIG. 18 is the cross-section of the fiber obtained in Example 8 (at a magnification of  $\times 10,000$ ).

[FIG. 19]

FIG. 19 is the surface of the fiber laminate obtained in Example 9 (at a magnification of  $\times 2,000$ ).

[FIG. 20]

FIG. 20 is the cross-section of the fiber obtained in Example 9 (at a magnification of  $\times 10,000$ ).

[FIG. 21]

FIG. 21 is the surface of the fiber laminate obtained in Comparative Example 1 (at a magnification of  $\times 2,000$ ).

[FIG. 22]

FIG. 22 is the cross-section of the fiber obtained in Comparative Example 1 (at a magnification of  $\times 10,000$ ).

[FIG. 23]

FIG. 23 is the surface of the fiber laminate obtained in Comparative Example 2 (at a magnification of  $\times 2,000$ ).

[FIG. 24]

FIG. 24 is the cross-section of the fiber obtained in Comparative Example 2 (at a magnification of  $\times 10,000$ ).

[FIG. 25]

FIG. 25 is the surface of the fiber laminate obtained in Comparative Example 3 (at a magnification of  $\times 2,000$ ).

[FIG. 26]

FIG. 26 is the cross-section of the fiber obtained in Comparative Example 3 (at a magnification of  $\times 10,000$ ).

[Description of Reference Numerals and Signs]

- 1 Ejection nozzle or nozzle
- 2 Solution to be spun
- 3 Tank holding solution to be spun
- 4 Electrode
- 5 Fibrous material-collection electrode
- 6 High voltage generator

[Designation of Document] Abstract

[Abstract]

[Problem] To provide fibers and a fiber laminate, which have large surface areas and large voids, and is capable of being controlled in hydrophilicity, suitable for a matrix of cell culture in the field of tissue engineering. At the same time, it is another object of the present invention to provide a production method of a porous fiber laminate which is capable of being controlled in hydrophilicity without needing complicated processes such as extraction and the like.

[Means for Resolution] A porous fiber comprising a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B), and having an average fiber diameter of 0.1-50  $\mu\text{m}$  and a void percentage of at least 5%, and a fiber laminate comprising the same. In addition, a method for producing the fiber laminate, including a step in which a solution is produced by dissolving a hydrophobic solvent-soluble polymer (A) and an organic compound having a plurality of hydroxyl groups (B) in a hydrophobic solvent, a step in which the solution is spun by electrospinning, and a step in which a fiber laminate accumulated on a collector is obtained.

[Selected Drawing] FIG. 1